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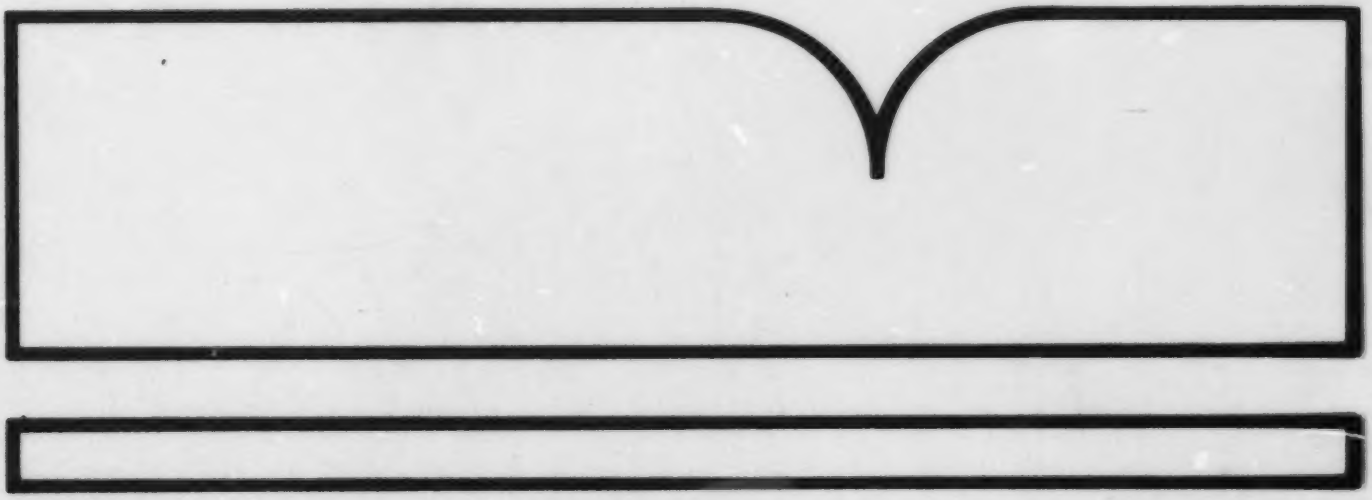

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16. ABSTRACT

The separate and combined effects of protein deprivation and benomyl [(methyl 1-butylcarbomoyl)2-benzimidazole carbamate] exposure were studied in the pregnant rat fed a diet containing 24% (control) or 8% (deficient) casein throughout gestation. Within each diet group, subgroups were gavaged at 31.2 mg/kg body weight with benomyl or corn-oil carrier only on d 7-16 or 7-21 of gestation. No effects on the skeleton were seen. Benomyl exposure in the last 2 wk in dams fed the 24% casein diet resulted in a high incidence of fetal brain anomalies. This effect did not occur in those with benomyl exposure during the period of organogenesis only and was reduced in groups fed the protein-deficient diet. Exposure to benomyl in the last 2 wk in the protein-deprived rat resulted in a decrease in the weight of the fetal heart in excess of that attributable to diet alone. Lungs were a smaller portion of body weight in fetuses of benomyl-treated dams in both diet groups. The teratogenic effect on the brain in animals exposed to benomyl in wk 2 and 3 of gestation suggests that screening for teratogenic effects during organogenesis only may be insufficient.

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JOURNAL ARTICLE

EFFECTS ON THE FETUS OF MATERNAL BENOMYL EXPOSURE IN THE PROTEIN-DEPRIVED RAT

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The separate and combined effects of protein deprivation and benomyl [(methyl 1-butylcarbomoyl)-2-benzimidazole carbamate] exposure were studied in the pregnant rat fed a diet containing 24% (control) or 8% (deficient) casein throughout gestation. Within each diet group, subgroups were gavaged at 31.2 mg/kg body weight with benomyl or corn-oil carrier only on d 7–16 or 7–21 of gestation. No effects on the skeleton were seen. Benomyl exposure in the last 2 wk in dams fed the 24% casein diet resulted in a high incidence of fetal brain anomalies. This effect did not occur in those with benomyl exposure during the period of organogenesis only and was reduced in groups fed the protein-deficient diet. Exposure to benomyl in the last 2 wk in the protein-deprived rat resulted in a decrease in the weight of the fetal heart in excess of that attributable to diet alone. Lungs were a smaller portion of body weight in fetuses of benomyl-treated dams in both diet groups. The teratogenic effect on the brain in animals exposed to benomyl in wk 2 and 3 of gestation suggests that screening for teratogenic effects during organogenesis only may be insufficient.

INTRODUCTION

Benomyl [(methyl 1-butylcarbomoyl)-2-benzimidazole carbamate] is a fungicide used on a variety of field crops and home plantings. The mechanism of action of benomyl's fungicidal properties lies in its ability to bind to tubulin (Davidse, 1973). However, in mammals, both benomyl and methyl 2-benzimidazole carbamate (MBC), its breakdown product in aqueous medium, are only weak binders of tubulin (Ireland et al., 1979; Friedman and Platzer, 1978).

In the case of animals given benomyl during pregnancy, however,

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the material is more toxic. Kavlock et al. (1982) described anomalies in neonates of Wistar rats gavaged at 31.2, 62.5, or 125 mg/kg · d on d 7–16 of gestation. Fetal anomalies have also been reported by Shtenberg and Torchinsky (1972), who administered 125 mg benomyl/kg on d 12 of gestation.

There is evidence of marked differences in the toxicity of pesticides in protein-deficient animals. Boyd (1982) reviewed studies of various pesticides in weanling and adult animals, but did not include benomyl. The effect of maternal protein deficiency on benomyl toxicity has not been thoroughly investigated. One study of the teratogenic effects of protein deficiency with benomyl exposure at 145 or 250 mg/kg body weight, on d 12 of gestation only, suggested that protein deficit had a modulating effect (Torchinsky, 1973).

The present study was undertaken to determine the role of protein deficiency in benomyl toxicity during pregnancy in rats.

METHODS

Virgin, female Sprague-Dawley rats (Bantin and Kingman, Fremont, Calif.) were housed individually in wire-bottom cages at constant temperature (22–24°C), with controlled lighting (7 a.m. to 7 p.m.). They were fed stock pellets (Purina Chow; Ralston Purina, St. Louis, Mo.) for at least 3 d to allow adjustment to the new environment, and were mated with normal males when female body weights were 180–200 g. Maternal body weights on d 0 of gestation did not differ significantly among the groups. The presence of copulation plugs was used to indicate that mating had occurred. The day following the night of mating is d 0 of pregnancy.

Throughout pregnancy, rats were fed either a semipurified diet containing 24% casein as the sole source of protein (control diet) or a diet containing 8% casein plus an additional 16% dextrose (protein-deficient diet). The composition of the control diet is given in Table 1. Tap water was provided ad libitum.

Each diet group was divided into four subgroups of six to eight each. One subgroup was gavaged with 31.2 mg benomyl (technical grade; E.I. DuPont de Nemours and Company, Wilmington, Del.) in 0.5 ml corn oil/kg body weight on d 7–16 of gestation. The second group was gavaged on d 7–21 of gestation. Daily doses of benomyl were based on body weight the same day, and suspensions were prepared daily with the aid of a magnetic stirrer. The 2 remaining subgroups were gavaged with the corn oil carrier only on d 7–16 or 7–21. The corn oil used for all groups was the same as that used in preparing the diets. The longer treatment period was used since previous work had shown that the effect on the fetus of maternal protein deficiency became evident primarily in stages of development that occur late in gestation.

Females were weighed daily, and food intake was measured daily. Scattered feed was collected, weighed, and deducted from the total.

On the afternoon of d 21 of gestation, young were taken by cesarean section under chloroform anesthesia and the following were noted for all young: number in the litter, location of each fetus in the uterus, fetal and placental weights, sex, and crown–rump length. Ratios of fetal weight to placental weight and weight to crown–rump length were calculated. Some rats delivered early. In these litters, young were counted, weighed, and examined for sex and external and internal congenital anomalies. Since their hydration status was unknown and would introduce an error, further study as described below on organ weights was not done for these early litters.

Weights of liver, kidneys, heart, brain, thymus, lung, and intestine were determined in three fetuses chosen at random from each litter.

TABLE 1. Composition of Diet

Component	Control (%)	Protein-deficient (%)
Glucose[a]	60	76
Casein[b]	24	8
Corn oil[c]	8	8
Salt mix[d]	6	6
Vitamin mix[e]	2	2
BHT	0.02	0.02

[a] Cerelose, Corn Products Co. (CPC International), New York, N.Y.

[b] ICN Nutritional Biochemical Co., Cleveland, Ohio.

[c] Mazola oil, Best Foods International, Inc., Englewood Cliffs, N.J.

[d] Expressed as g/100 g salt mix: $CaCO_3$, 15.5; Ca_2HPO_4, 41.64; K_2HPO_4, 29.48; NaCl, 4.69; $MgSO_4$, 7.296; $CrK(SO_4)_2 \cdot 12H_2O$, 0.01061; $CuSO_4 \cdot 5H_2O$, 0.07240; KI, 0.00072; $FeSO_4 \cdot 7H_2O$, 0.64487; $MnSO_4 \cdot H_2O$, 0.56563; $ZnCO_3$, 0.08475; Na_2SeO_3, 0.00805; $Na_2MoO_4 \cdot H_2O$, 0.02948; and $KF \cdot 2H_2O$, 0.01842.

[e] Expressed as g/100 g mix (except as noted): inositol, 2.5; ascorbic acid, 0.5; Ca pantothenate, 250 mg; thiamine HCl, 150 mg; pyridoxine HCl, 150 mg; nicotinic acid, 150 mg; menadione, 125 mg, riboflavin, 50 mg; *p*-aminobenzoic acid, 50 mg; biotin, 1.25 mg; folic acid, 3 mg; vitamin E (Rovimix E-50%), 1.19; vitamin A (Rovimix A-250), 273 mg; cholecalciferol (Rovimix-AD_3 325/325), 23 mg; vitamin B-12 + mannitol (Merck), 15 mg; glucose, 89.5; and choline Cl (70% solution), 7.15 (Rovimix, Hoffman-LaRoche, Nutley, N.J.; Merck & Co., Rahway, N.J.).

Organs were lightly blotted before weighing. Ratios of organ weight to body weight were calculated. Intestines were not emptied before weighing, in order to avoid weight loss from dehydration. Intestinal weights, therefore, include the weight of the meconium.

The skeletons of three additional fetuses from each litter were stained with Alizarin red and examined for presence or absence of ossification sites in the axial and appendicular skeletons using the method of Dawson (1926). In addition, measurements of skull length, spine length, pelvic length, ilium length, biiliac width, and biacetabular width were made from X-ray photographs of Alizarin-stained skeletons following the procedures of Dickerson and Hughes (1972). All fetuses were examined externally for gross congenital anomalies. Three to six fetuses, chosen at random from each litter, were examined internally for anomalies by the method of Monie et al. (1965).

The occurrence of brain anomalies in the d 7–21 benomyl-treated group, but not in the d 7–16 treated group, was unexpected. As a consequence, additional litters were added to the d 7–21 benomyl-exposed groups fed 8% and 24% casein diets in order to confirm results seen with the original groups. Since the earlier results were confirmed, the data were combined. This replicate experiment was confined to the study of the occurrence of brain anomalies.

Most data were analyzed by two-way analysis of variance with interactions (Helwig and Council, 1979). The litter was used as the unit for analysis. As an exception, data on the incidence of congenital anomalies was analyzed with the chi-square test (Croxton, 1953).

RESULTS

The pregnant protein-deficient rat gained less weight over the total course of gestation than did those fed the 24% casein diet (Table 2), while feed intake was unaffected by diet. Thus, feed efficiency, the weight gain per gram of feed intake, was decreased in the protein-deprived groups. None of these parameters was affected by benomyl exposure. Neither diet nor toxin exposure significantly affected the number of implantation sites or the number of live young per litter observable at 21 d of gestation; however, not all dams fed the restricted diet showed evidence of pregnancy at 21 d of gestation.

At 21 d of gestation, placentas and fetuses of protein-deficient dams were smaller than those of control dams, as indicated by reduced body weight and crown–rump length. (Table 3). The ratio of weight to crown–rump length was also reduced. Crown–rump length was reduced in the pups of dams exposed to benomyl on d 7–21, but exposure only during organogenesis had no significant effect. The ratio of weight to length was reduced in the young of protein-deprived dams

TABLE 2. Effects on the Pregnant Rat of Benomyl Exposure[a] and Protein Deprivation

Parameter	Carrier only		Benomyl exposure		Significance of differences p[b]		
	24% Casein	8% Casein	24% Casein	8% Casein	D	B	I
Exposure d 7–16							
n	7	6	7	7			
Maternal weight change	177 ± 6[c]	65 ± 10	150 ± 14	63 ± 9	0.001	NS	NS
Feed intake (g)	371 ± 12	347 ± 21	360 ± 16	378 ± 19	NS	NS	NS
Feed efficiency[d]	0.47 ± 0.01	0.19 ± 0.02	0.42 ± 0.03	0.17 ± 0.02	0.001	NS	NS
Exposure d 7–21							
n	8	6	7	7			
Maternal weight change	177 ± 7	69 ± 12	138 ± 21	69 ± 8	0.001	NS	NS
Feed intake (g)	387 ± 13	372 ± 16	422 ± 15	369 ± 22	NS	NS	NS
Feed efficiency[d]	0.46 ± 0.02	0.19 ± 0.02	0.33 ± 0.03	0.19 ± 0.02	0.001	NS	NS

[a] At 31.2 mg in oil/kg body weight · d, gavage-fed.
[b] D = diet; B = benomyl; I = interaction; NS = not significant.
[c] Mean ± SEM.
[d] Weight gain, g/g feed intake.

TABLE 3. Effects on Placental and Fetal Size of Benomyl Exposure[a] in the Protein-Deprived Rat

Parameter	Carrier only		Benomyl exposure		Significance of differences p[b]		
	24% Casein	8% Casein	24% Casein	8% Casein	D	B	I
Exposure d 7–16							
n	7	6	6	7			
Placental weight (g)	0.94 ± 0.04[c]	0.69 ± 0.09	0.99 ± 0.10	0.60 ± 0.05	<0.002	NS	NS
Fetal body weight (g)	5.49 ± 0.11	3.83 ± 0.12	5.59 ± 0.30	3.88 ± 0.21	<0.0001	NS	NS
Crown–rump length (cm)	4.00 ± 0.03	3.61 ± 0.09	4.16 ± 0.14	3.68 ± 0.11	0.001	NS	NS
Weight–length ratio	1.37 ± 0.03	1.07 ± 0.09	1.36 ± 0.04	1.07 ± 0.05	<0.0001	NS	NS
Exposure d 7–21							
n	7	7	5	6			
Placental weight (g)	0.87 ± 0.05	0.60 ± 0.03	0.76 ± 0.05	0.61 ± 0.04	<0.0001	NS	NS
Fetal body weight (g)	5.36 ± 0.09	3.85 ± 0.20	4.62 ± 0.15	3.81 ± 0.18	<0.0001	NS	NS
Crown–rump length (cm)	3.96 ± 0.03	3.64 ± 0.06	3.68 ± 0.11	3.50 ± 0.07	<0.0002	<0.05	NS
Weight–length ratio	1.36 ± 0.03	1.06 ± 0.04	1.26 ± 0.04	1.08 ± 0.06	<0.0001	NS	NS

[a] At 31.2 mg in oil/kg body weight · d, by gavage.
[b] D = diet; B = benomyl; I = interaction; NS = not significant.
[c] Mean ± SEM.

but was unaffected by toxin exposure. Fetal–placental weight ratios were unaffected by either diet or benomyl exposure.

All fetal organs examined were reduced in size in the protein-deprived groups (Table 4). With a shorter term of benomyl exposure, none of the organs was significantly altered, nor was there any interaction between toxin exposure and protein deprivation with short-term exposure. In the case of the groups with the longer exposure, lung weights were reduced in benomyl-exposed groups while weights of other organs were unaffected. There was no evidence of interaction between the diet and toxin exposure.

Organ weights relative to body weights are shown in Table 5. Ratios of liver, intestine, lung, or thymus weight to body weight were unaffected by diet or by d 7–16 benomyl exposure. Relative kidney weight was reduced in young of protein-deprived dams compared to controls. Relative heart weight tended to be greater in protein-deprived fetuses, but the difference was not statistically significant ($p < 0.07$). Relative brain weight was increased in the young of protein-deprived dams. Relative heart weight also tended to be larger in benomyl-exposed groups, but again the difference was not statistically significant ($p < 0.055$), and there was no interaction between the treatments.

Examination of Alizarin-stained fetal skeletons demonstrated that the number of ossification sites was unaffected by either maternal nutritional deficiency or exposure to benomyl. Skeletal measurements also did not differ significantly. Detailed data are therefore not given.

The results of examination of the fetuses for congenital anomalies are shown in Table 6. Some stillbirths and a few congenital anomalies appeared in the control diet group exposed to benomyl, but there were a larger number and variety of congenital anomalies in the group exposed to benomyl on d 7–21 than in those exposed on d 7–16. In particular, the longer exposure to benomyl had a deleterious effect on the nervous system, shown by a marked incidence of hydrocephaly, exencephaly, or both, along with some anophthalmia and microophthalmia. The number affected was higher in young of control dams than in young of deficient dams. Not all litters were affected and not all pups in affected litters showed anomalies.

DISCUSSION AND CONCLUSIONS

The data from this study confirm results of previous investigations of the effects of protein deprivation in pregnancy indicating lower maternal weight gain, decreased mean fetal and placental weights, and decreased fetal length as well as a reduced number of litters produced (Zeman, 1968; Hastings-Roberts and Zeman, 1977). Ratios of organ weight to body weight again suggest, as previously shown (Zeman, 1967), that the brain and heart weights are somewhat protected—that

TABLE 4. Fetal Organ Weights (mg) in Fetuses of the Benomyl-Exposed[a] and Protein-Deficient Pregnant Rat

Organ	Treatment				Significance of differences p^b		
	Carrier only		Benomyl exposure				
	24% Casein	8% Casein	24% Casein	8% Casein	D	B	I
Exposure d 7–16							
n	6	4	7	7			
Liver	350.01 ± 32.32[c]	228.96 ± 16.35	394.38 ± 27.73	275.80 ± 29.30	<0.0001	NS	NS
Kidney	59.66 ± 3.40	38.35 ± 1.80	62.16 ± 5.36	37.39 ± 2.81	<0.0001	NS	NS
Intestine	197.78 ± 14.86	145.07 ± 5.25	220.33 ± 20.08	138.30 ± 8.43	<0.001	NS	NS
Heart	40.35 ± 3.82	31.83 ± 3.23	48.27 ± 4.85	40.11 ± 3.44	<0.07	<0.07	NS
Lung	134.04 ± 3.57	92.86 ± 3.80	124.14 ± 6.02	90.60 ± 7.38	<0.0001	NS	NS
Thymus	12.64 ± 1.35	9.58 ± 0.86	11.41 ± 0.85	8.49 ± 0.72	<0.01	NS	NS
Brain	213.92 ± 3.87	188.50 ± 2.50	218.65 ± 9.71	181.92 ± 5.65	<0.001	NS	NS
Exposure d 7–21							
n	7	7	7	6			
Liver	422.28 ± 10.29	266.79 ± 17.47	375.52 ± 16.90	261.52 ± 27.85	<0.0001	NS	NS
Kidney	57.45 ± 2.52	37.78 ± 2.23	55.59 ± 3.42	36.38 ± 3.85	<0.0001	NS	NS
Intestine	203.55 ± 9.40	136.23 ± 10.26	176.95 ± 7.20	137.40 ± 5.91	<0.0001	NS	NS
Heart	46.67 ± 2.15	46.11 ± 3.04	47.67 ± 1.36	37.00 ± 1.27	<0.01	NS	NS
Lung	138.06 ± 6.94	101.70 ± 8.40	114.95 ± 3.99	85.75 ± 7.99	<0.0001	<0.01	NS
Thymus	11.00 ± 1.13	9.37 ± 1.01	10.02 ± 0.69	8.30 ± 1.03	NS	NS	NS
Brain	210.74 ± 2.66	188.26 ± 3.72	208.08 ± 9.54	188.49 ± 5.17	<0.02	NS	NS

[a] At 31.2 mg in oil/kg body weight · d, by gavage.
[b] D = diet; B = benomyl; I = interaction; NS = not significant.
[c] Mean ± SEM.

TABLE 5. Effects on Relative Fetal Organ Weights of Benomyl Exposure[a] and Protein Deficiency in the Pregnant Rat[b]

Organ	Treatment				Significance of differences p[c]		
	Carrier only		Benomyl exposure				
	24% Casein	8% Casein	24% Casein	8% Casein	D	B	I
Exposure d 7–16							
n	6	4	7	7			
Liver	6.373 ± 0.607	5.963 ± 0.475	7.168 ± 0.628	6.960 ± 0.401	NS	NS	NS
Kidney	1.083 ± 0.063	0.996 ± 0.032	1.108 ± 0.055	0.958 ± 0.046	<0.05	NS	NS
Intestine	3.551 ± 0.183	3.754 ± 0.149	3.919 ± 0.195	3.545 ± 0.124	NS	NS	NS
Heart	0.740 ± 0.077	0.835 ± 0.091	0.868 ± 0.731	1.033 ± 0.075	<0.07	<0.055	NS
Lung	2.442 ± 0.067	2.426 ± 0.111	2.251 ± 0.129	2.313 ± 0.112	NS	NS	NS
Thymus	0.232 ± 0.029	0.249 ± 0.018	0.204 ± 0.009	0.219 ± 0.015	NS	NS	NS
Brain	3.878 ± 0.095	4.936 ± 0.107	3.965 ± 0.050	4.733 ± 0.198	<0.0001	NS	NS
Exposure d 7–21							
n	7	7	7	6			
Liver	7.891 ± 0.154	6.958 ± 0.413	7.391 ± 0.333	6.811 ± 0.413	<0.05	NS	NS
Kidney	1.072 ± 0.044	0.982 ± 0.029	1.102 ± 0.096	0.945 ± 0.072	<0.07	NS	NS
Intestine	3.769 ± 0.135	3.539 ± 0.184	3.446 ± 0.097	3.621 ± 0.099	NS	NS	NS
Heart	0.874 ± 0.056	1.224 ± 0.128	0.995 ± 0.055	0.982 ± 0.055	<0.05	NS	<0.05
Lung	2.569 ± 0.072	2.632 ± 0.084	2.347 ± 0.073	2.245 ± 0.089	NS	<0.02	NS
Thymus	0.206 ± 0.022	0.244 ± 0.026	0.228 ± 0.017	0.216 ± 0.018	NS	NS	NS
Brain	3.916 ± 0.086	4.955 ± 0.198	4.424 ± 0.236	4.997 ± 0.209	<0.05	NS	NS

[a] At 31.2 mg in oil/kg body weight · d, by gavage.
[b] Results given as [(g organ weight)/(g body weight)] × 10^2, mean ± SEM.
[c] D = diet; B = benomyl; I = interaction; NS = not significant.

TABLE 6. Congenital Anomalies and Stillbirths in Fetuses of Rats Malnourished and Exposed to Benomyl[a] (BML) During Gestation

Diet and gavage feeding	Pups examined/litter	Anomalies and stillbirths[b]	
		Brain and eyes	Other
Exposure d 7–16			
24% + oil	21/6	0	0
8% + oil	28/6	0	0
24% + BML	50/7	1/1[c]	7/3[d,e]
8% + BML	37/7	0	0
Exposure d 7–21			
24% + oil	42/7	0	0
8% + oil	42/7	0	1/1[f]
24% + BML	83/15	18/7[g,h]	9/7[e,i]
8% + BML	45/17	5/3[h]	3/2[j]

[a] At 31.2 mg/kg body weight.
[b] Pups affected/litters affected.
[c] Enlarged ventricles.
[d] Five stillbirths, one short tail, one clubbed foot.
[e] Significantly different from 8% + BML groups, $p < 0.02$.
[f] Bilateral cataracts.
[g] Eleven hydrocephaly; four exencephaly; two anophthalmia; one microophthalmia.
[h] Significantly different from 24% + oil group, $p < 0.05$.
[i] Four hydroureter, two absent pinna; one cleft lip; one short, kinky tail.
[j] Hydrocephaly.
[k] Significantly different from 8% + oil group, $p < 0.05$.
[l] Kinky tails.

is, they are not as affected as total body weight in maternal protein malnutrition.

Feed efficiency in the rat has been previously shown to increase during pregnancy but decreases if the pregnant rat is fed a protein-deficient diet (Zeman, 1983). The increase may be a physiological adaptation to pregnancy, the specific mechanisms of which are unclear. Naismith (1973) suggested that adaptation failure could be the result of reduced action of progesterone or adrenal cortical hormones. The protein-deficient diet in this work appeared to interfere with this adaptation, which was unaffected by benomyl.

The benomyl dose chosen was marginal for teratogenic effects in the rat according to Kavlock et al. (1982). They reported 7 abnormal fetuses in 24 adequately fed litters that were gavaged on d 7–16 with the same dose used in this study. The higher incidence of abnormalities seen in the present study may be attributed to the differences in the method of calculating the dose. Kavlock et al. (1982) based all doses on the maternal weight on gestational d 4. In this study, females were weighed daily and total doses changed accordingly, resulting in a higher doses, as total weight increased, but the dose per kilogram of

weight remained constant. It is estimated that blood constitutes approximately 5% of body weight. Thus, benomyl given in proportion to body weight is assumed to maintain a constant concentration in the blood as body size and blood volume expand.

While the shorter-term (d 7–16) benomyl exposure had little effect on relative organ size, the data indicate that longer exposure (d 7–21) tended to result in lower absolute and relative lung size. Previous work (Zeman, 1967) suggested that the fetal heart and brain were protected from the effects of maternal protein deprivation. This relationship is confirmed in the groups administered oil only in this study. However, the absence of this same relationship in the benomyl-exposed groups suggests that benomyl exposure may interfere with this protective effect.

The nervous-system anomalies seen in the benomyl-exposed group constitute the most serious effects seen in these young. It is of great interest that there was almost no evidence of these anomalies in the short-term exposed group, particularly in view of the early development of the central nervous system. It is possible that anomalies existed in the d 7–16 exposed animals that were not sufficiently severe to be visible with the methods used. In animals exposed to ethylenethiourea (ETU), a structurally related compound, hydrocephalus was produced that was not grossly evident until a week after birth, even though microscopic changes occur much earlier (Tryphonas and Khera, 1980). These authors report evidence in ETU-exposed pups of necrosis of the paraventricular epithelium and enlargement of the ventricular system without evidence of obstruction of the aqueduct. In this work aqueduct obstruction remains a possibility. It has also been suggested that exencephaly may be evidence of the rupture of the roof plate as a consequence of the hydrocephaly (Gardner, 1977). The mechanism of the brain anomalies seen and the temporal relationships between benomyl exposure and the anomalies produced in this work are under investigation. The data available to date on the brain, and to some extent on the lungs, suggest that fetal exposure to a toxic substance during organogenesis only, defined in the rat as approximately d 8–16 (Rugh, 1968), is an inadequate screening protocol for detection of teratogenic effects.

The reduced number of teratogenic effects on the central nervous system in the protein-deficient, benomyl-exposed group suggests that protein deficit may have a modulating, but not preventative, effect on the central-nervous-system teratogenicity of benomyl. This would be in agreement with similar results from Torchinsky (1973). The mechanism of the modulating effect is unknown. Protein malnutrition increases the toxicity of many xenobiotics (Boyd, 1982). These deficiencies impair hepatic metabolizing enzymes such as cytochrome P-450, NADPH oxidase, and NADPH-cytochrome c reductase (Dauterman, 1982). Thus,

when the metabolic reaction is a detoxication reaction, toxicity could be expected to be increased in the protein-deprived animal. Alternatively, if the microsomal reaction results in the formation of the toxic compound, the consequence of malnutrition can be a decrease in toxicity. Dietary protein may also affect conjugation reactions (Dauterman, 1982).

In pregnancy, conjugation of xenobiotics decreases as progesterone and pregnanediol, inhibitors of glucuronyltransferase, increase. Microsomal monooxygenase activity in pregnancy also has been shown to decrease (Dauterman, 1982). Further study of the metabolism of benomyl in the maternal–fetal organism is required to answer the question of its action in pregnancy.

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